Research Paper | Pharmacognosy | E-ISSN No : 2454-9916 | Volume : 10 | Issue : 10 | October 2024

IN-VITRO ASSESSMENT OF TOTAL PHENOLIC AND FLAVONOID CONTENT OF IXORA POLYANTHA WIGHT.

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ABSTRACT

The determination of total phenolic and flavonoid content in *Ixora polyantha* Wight is crucial for assessing its pharmacological potential. The ethanolic extract exhibited a high phenolic content of 113.5 µg/ml, indicating the plant's capacity as a significant source of natural antioxidants that neutralize free radicals and prevent oxidative cellular damage. This finding aligns with the plant's traditional uses in treating inflammation, wounds, and oxidative stress-related disorders, supported by a strong correlation ($R^2 = 0.9977$) confirming the reliability of the Folin-Ciocalteu method for quantification. Additionally, the extract displayed a moderate flavonoid content of 56.6 µg/ml, further supporting its antioxidant, anti-inflammatory, and antimicrobial properties. The high correlation coefficient ($R^2 = 0.9996$) from the Aluminum Chloride Colorimetric method validates the accuracy of these measurements, essential for standardizing herbal formulations. Collectively, the identified phenolic and flavonoid contents position *Ixora polyantha* as a promising medicinal plant, suggesting potential applications in managing oxidative stress, inflammatory diseases, and infections. Further studies are necessary to isolate and characterize specific bioactive compounds, enhancing the development of standardized herbal formulations for clinical use. These findings underscore the plant's significance in traditional medicine and its promise for modern therapeutic applications.

KEYWORDS: *Ixora polyantha* Wight., Total Phenolic Content, Total Flavonoid Content, Herbal Medicines

INTRODUCTION

Healing with medicinal plants is an old treatment method as old as mankind itself. Nature has been a source of medicinal agents for thousands of years and an impressive number of modern drugs have been isolated from natural sources, many based on their use in traditional medicine [1]. The connection between human and their search for drugs in nature dates from ancient times of which there are enormous evidence from different sources (written documents, preserved monuments, and even original plant medicines) [2,3]. India officially endorses over 3000 plants for their medicinal value. It is generally estimated that over 6000 plants in India are used in traditional, folk and herbal medicine. Herbal medicine is fast emerging treatment as an alternative to available synthetic drugs for treatment of various diseases, possibly due to reduced side effects and lower costs [4,5]. Several chemical compounds have been isolated from medicinal plants. More than 70% of the world's population now depends on the traditional medicinal system, otherwise known as complementary or alternative systems of medicine [6,7].

Ixora polyantha Wight, a plant species known for its medicinal properties, is part of the Rubiaceae family. Traditionally, it has been used for various therapeutic purposes, including anti-inflammatory, antioxidant, and wound-healing treatments. The phytochemical constituents, particularly flavonoids and phenolics, play a crucial role in these biological activities. Determining the total flavonoid and phenolic content in *Ixora polyantha* is a fundamental step to validate its therapeutic potential and explore its utility in pharmaceutical formulations [8,9].

Flavonoids and phenolics are secondary metabolites in plants known for their significant antioxidant properties [10]. These compounds scavenge free radicals and contribute to various pharmacological effects, such as anti-inflammatory, antimicrobial, and antidiabetic activities. In particular, flavonoids are a group of polyphenolic compounds that are widely distributed in plants, contributing to their color, flavor, and disease resistance. They have gained attention due to their potential health benefits, including reducing the risk of chronic diseases such as cardiovascular diseases, cancers, and diabetes. Phenolic compounds, on the other hand, include a wide range of substances such as phenolic acids, tannins, and lignins, which also contribute to the plant's defense mechanism and antioxidant activity [11].

The quantification of flavonoids and phenolics in plant extracts provides insights into the plant's medicinal potential. This is particularly important for plants like Ixora polyantha, where traditional knowledge and modern pharmacological studies intersect. Several analytical methods have been developed to estimate the total flavonoid and phenolic content in plant materials, with spectrophotometric techniques being among the most common.

Method for Determining Total Flavonoid Content

The determination of total flavonoid content in *Ixora polyantha* is typically carried out using colorimetric methods, with the aluminum chloride method being the most widely used. This method is based on the formation of a flavonoid-aluminum complex, which produces a yellow color. The intensity of this color, measured using a spectrophotometer at a specific wavelength (usually 415 nm), is directly proportional to the flavonoid concentration in the extract. The results are expressed in terms of a standard flavonoid compound, such as quercetin or rutin, allowing for comparison across different samples [12].

Method for Determining Total Phenolic Content

Similarly, the total phenolic content of *Ixora polyantha* is determined using the Folin-Ciocalteu method, which is a widely accepted and reliable colorimetric method. This method involves the reduction of the Folin-Ciocalteu reagent by phenolic compounds under alkaline conditions, leading to the formation of a blue complex that can be quantified spectrophotometrically at 765 nm. The results are typically expressed in terms of a standard phenolic compound, such as gallic acid.

Both flavonoid and phenolic assays involve preparing plant extracts using solvents such as methanol, ethanol, or water. The choice of solvent depends on the solubility of the phenolic and flavonoid compounds in the plant material. Following extraction, the plant material is subjected to the respective assays to determine the concentration of these compounds.

The determination of total flavonoid and phenolic content in *Ixora polyantha* is not only essential for understanding its pharmacological potential but also for developing standardization protocols for its use in herbal medicine or pharmaceutical formulations. These compounds contribute significantly to the plant's therapeutic properties, including its antioxidant, anti-inflammatory, and wound-healing effects, making their quantification a critical aspect of research into the pharmacognosy of this plant [13].

METHODOLOGY

1. Preparation of plant extract:

The *Ixora polyantha* Wight. plant leaves was shade dried and coarsely powdered. 90 g of coarsely powdered leaves was packed in Soxhlet apparatus and extracted by using ethanol (approx. 2 days). The extract was collected and filtered through Whatman No. 1 filter paper and concentrated by evaporation and stored in air tight container. The percentage yield of extracts was calculated

$$\text{Percentage yield} = \frac{\text{Weight of crude drug (g)}}{\text{Weight of plant material taken (g)}} \times 100$$

2. Determination of Total Phenolic Content

Phenolic compounds are a large group of secondary metabolites found in plants, recognized for their antioxidant, anti-inflammatory, and antimicrobial properties. Phenolics, including phenolic acids, flavonoids, tannins, and lignins, contribute significantly to the overall therapeutic benefits of medicinal plants. These compounds function as potent antioxidants due to their ability to neutralize free radicals and inhibit oxidative stress, which is often linked to various diseases, such as cancer, cardiovascular disorders, and diabetes. The quantification of total phenolic content (TPC) provides valuable insights into a plant's antioxidant capacity and therapeutic potential [14].

Methodology for Determining Total Phenolic Content

The Folin-Ciocalteu method is the most commonly used spectrophotometric technique for determining TPC. This assay is based on the reduction of the Folin-Ciocalteu reagent by phenolic compounds present in the sample, resulting in the formation of a blue-colored complex that can be measured using a spectrophotometer..

1. **Reaction with Folin-Ciocalteu Reagent:** A known volume of the extract is mixed with the Folin-Ciocalteu reagent. This reagent contains phosphomolybdic and phosphotungstic acid, which react with phenolic hydroxyl groups under alkaline conditions.
2. **Development of Blue Color:** Upon reaction, the phenolic compounds reduce the Folin-Ciocalteu reagent, resulting in a blue-colored complex. Sodium carbonate is added to maintain alkaline conditions, which are necessary for the reaction to proceed.
3. **Spectrophotometric Measurement:** The intensity of the blue color is measured using a spectrophotometer at a wavelength of 765 nm. The intensity is proportional to the amount of phenolic compounds present in the sample.
4. **Quantification:** The TPC is expressed in terms of a standard phenolic compound, typically gallic acid, which is used to create a standard calibration curve. The results are usually presented as milligrams of gallic acid equivalents (GAE) per gram of plant extract.

The Folin-Ciocalteu assay is widely used due to its simplicity and reproducibility. However, it is not specific to phenolics alone, as other reducing agents (such as ascorbic acid or reducing sugars) may also contribute to the reaction, potentially leading to an overestimation of the phenolic content [10].

3. Determination of Total Flavonoid Content

Flavonoids are a subclass of phenolic compounds known for their diverse biological activities, including antioxidant, anti-inflammatory, antiviral, and antidiabetic effects. Flavonoids are categorized into several subclasses, such as flavonols, flavones, flavanones, and isoflavonoids, each with unique structural characteristics and health benefits. They play a crucial role in plant defense mechanisms, particularly in protecting against UV radiation and pathogens.

Methodology for Determining Total Flavonoid Content

The aluminum chloride (AlCl3) colorimetric method is the most common assay for determining total flavonoid content (TFC). This method involves the formation of a flavonoid-aluminum complex, which produces a yellow color that can be quantified spectrophotometrically.

1. **Formation of Flavonoid-Aluminum Complex:** The plant extract is mixed with aluminum chloride, which

reacts specifically with the hydroxyl groups on flavonoids to form a complex. This reaction typically takes place in the presence of an acidic medium (often acetic acid or hydrochloric acid).

2. **Development of Yellow Color:** The reaction between flavonoids and aluminum chloride produces a yellow complex. The intensity of this color correlates with the flavonoid concentration in the sample.
3. **Spectrophotometric Measurement:** The intensity of the yellow color is measured using a spectrophotometer at a wavelength of 415 nm. This wavelength is optimal for detecting the flavonoid-aluminum complex.
4. **Quantification:** The TFC is expressed in terms of a standard flavonoid compound, usually quercetin or rutin. A calibration curve is prepared using standard solutions of quercetin or rutin, and the flavonoid content is expressed as milligrams of quercetin or rutin equivalents per gram of dry plant material or extract.

This method is preferred due to its simplicity and specificity for flavonoids. However, it may not differentiate between different types of flavonoids, and other compounds, such as phenolic acids, may interfere with the assay.

Importance of Determining Total Phenolic and Flavonoid Content

The quantification of total phenolic and flavonoid content is essential for several reasons:

1. **Antioxidant Activity:** Both phenolics and flavonoids are strong antioxidants, neutralizing free radicals and preventing oxidative damage to cells. Measuring their content helps in assessing the plant's antioxidant capacity, which is crucial for understanding its therapeutic potential.
2. **Health Benefits:** Flavonoids and phenolics have been associated with a wide range of health benefits, including reducing the risk of chronic diseases such as cancer, cardiovascular disease, diabetes, and neurodegenerative disorders. By quantifying these compounds, researchers can evaluate the plant's potential in preventing or treating these conditions.
3. **Standardization:** The determination of these bioactive compounds is critical for the standardization of herbal medicines and formulations. Ensuring consistent levels of phenolics and flavonoids ensures the efficacy and safety of plant-based medicines.
4. **Quality Control:** In the pharmaceutical and nutraceutical industries, quantifying phenolic and flavonoid content is part of the quality control process. It ensures that products meet regulatory requirements and maintain their therapeutic effectiveness.

The determination of total phenolic and flavonoid content provides a crucial understanding of the bioactive potential of plants like *Ixora polyantha* Wight. These compounds contribute to the plant's pharmacological properties, making their quantification an essential step in medicinal plant research, pharmaceutical applications, and the development of herbal formulations. By employing methods such as the Folin-Ciocalteu and aluminum chloride assays, researchers can accurately assess the antioxidant potential and therapeutic efficacy of medicinal plants [11].

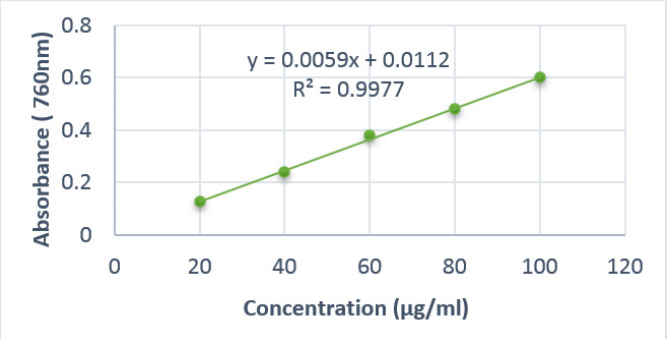
RESULT

Determination of Total Phenolic Content: FOLIN-CIOCALTEU METHOD

Concentration(µg/ml)	Absorbance (760nm)
20	0.129
40	0.239
60	0.381
80	0.481
100	0.599
Plant extract	0.290

Table 01: Absorbance of Standard Gallic acid and extract at 760nm

Calibration curve of Standard Gallic acid



Graph 01: Calibration curve of Standard Gallic acid

The results presented in the image pertain to the quantitative estimation of the total phenolic content in the ethanolic leaf extract of *Ixora polyantha* Wight, using the Folin-Ciocalteu method. The method relies on the reaction between phenolic compounds and the Folin-Ciocalteu reagent, which produces a blue complex whose absorbance is measured at 750 nm. Based on the absorbance values obtained at different concentrations (20, 40, 60, 80, and 100 µg/ml), a standard calibration curve was generated. The data shows a linear relationship between concentration and absorbance, with the regression equation given as $y = 0.0059x + 0.012$, and the R^2 value of 0.9977 indicating a high degree of correlation. The absorbance of the plant extract was measured to be 0.29 at 750 nm. By substituting this absorbance value into the calibration curve equation, the total phenolic content of the ethanolic extract of *Ixora polyantha* leaves was calculated to be 113.5 µg/ml, expressed in terms of gallic acid equivalents (GAE). This result indicates that the extract contains a significant amount of phenolic compounds, which could be responsible for the plant's observed antioxidant and therapeutic properties.

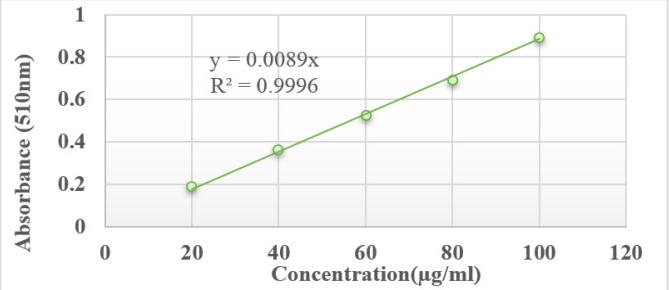
Determination of Total Flavonoid Content
ALUMINIUM CHLORIDE COLORIMETRIC METHOD

Absorbance of Standard Quercetin and extract at 510nm

Concentration(µg/ml)	Absorbance (510nm)
20	0.189
40	0.365
60	0.525
80	0.693
100	0.894
Plant extract	0.494

Table 02: Absorbance of Standard Quercetin and extract at 510nm

Calibration curve of Standard Quercetin



Graph 02: Calibration curve of Standard Quercetin

This method is based on the complex formation between flavonoids and aluminum chloride, which produces a yellow coloration measurable at 510 nm. The table shows the absorbance values at different concentrations (20, 40, 60, 80, and 100 µg/ml), which were used to construct a standard calibration curve. The linear relationship between absorbance and concentration is represented by the equation y = 0.0088x + 0.012, with a high correlation coefficient (R^2 = 0.9996), indicating a strong correlation between concentration and absorbance. The absorbance of the plant extract was recorded at 0.494 at 510 nm. Based on the calibration curve, the total flavonoid content in the ethanolic extract of *Ixora polyantha* leaves was calculated to be 56.6 µg/ml, expressed as quercetin equivalents.

DISCUSSION

The determination of total phenolic and flavonoid content in *Ixora polyantha* Wight is essential for understanding its Pharmacological potential as a good Antioxidant and Anti Inflammatory activity so the ethanolic extract of *Ixora polyantha* Wight. posses good Anti-diabetic with good wound healing activity. The ethanolic extract revealed a high phenolic content of 113.5 µg/ml, indicating the plant's capacity as a source of natural antioxidants, which is critical for neutralizing free radicals and preventing oxidative damage to cells. This finding supports the traditional uses of *Ixora polyantha* in treating inflammation, wounds, and oxidative stress-related disorders, with a strong correlation (R^2 = 0.9977) confirming the reliability of the Folin-Ciocalteu method for quantification. In addition, the extract demonstrated a moderate total flavonoid content of 56.6 µg/ml, reinforcing its role in antioxidant, anti-inflammatory, and antimicrobial activities. The high correlation coefficient (R^2 = 0.9996) from the Aluminum Chloride Colorimetric method further validates the accuracy of these measurements, which are vital for standardizing herbal formulations. Together, the phenolic and flavonoid contents highlight *Ixora polyantha* as a promising medicinal plant, suggesting its potential in managing oxidative stress, inflammatory diseases, and various other infections. Further research is warranted to isolate and characterize the specific bioactive compounds, which could enhance the development of standardized herbal formulations for clinical applications. Overall, these findings underscore the plant's value in traditional medicine and its potential for modern therapeutic use.

CONCLUSION

The high total phenolic content of 113.5 µg/ml and moderate flavonoid content of 56.6 µg/ml in *Ixora polyantha* Wight highlight its significant pharmacological potential as a natural source of antioxidants. These findings support the plant's traditional medicinal uses for treating inflammation, wounds, and oxidative stress-related disorders. The strong correlation coefficients (R^2 = 0.9977 for phenolics and R^2 = 0.9996 for flavonoids) confirm the reliability of the methods used for quantification, ensuring the accuracy necessary for developing standardized herbal formulations. The combined antioxidant, anti-inflammatory, and antimicrobial properties of these compounds reinforce the potential of *Ixora polyantha* in modern therapeutic applications. Further research aimed at isolating and characterizing specific bioactive compounds will be essential for enhancing its clinical efficacy and supporting its role in contemporary medicine. Overall, these results underscore the value of *Ixora polyantha* in both traditional and modern health practices.

AKNOWLEDGEMENT

I to express my heartfelt gratitude to Nehru College of Pharmacy for providing the excellent facilities necessary for conducting my research. My sincere thanks to Prof. Dr. Pramodini for her invaluable guidance and insightful feedback throughout the research process. I am also deeply grateful to Dr. M. Rajan for his unwavering support and dedication to our study. His assistance and thoughtful contributions greatly enhanced the quality of this research. This work would not have been possible without their collective support and commitment.

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